Colchicine Treatment on Sterile Hybrid Sundews

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Keywords: cultivation: *Drosera*, hybrids — genetics: colchicine.

Introduction

Sundew hybrids between species with different chromosome numbers are usually sterile. With the treatment of colchicine some of these can be made fertile. The process is fairly simple—I am merely a hobbyist grower but have been able to produce fertile plants without a laboratory. It requires much dedication since it will take several months to nurture and raise treated plants, and longer still to select the most vigorous individuals. Some of you might use the methods I describe to make your own fascinating hybrids fertile. If so, take note that colchicine is toxic and possibly carcinogenic. You must take safety measures when using this chemical, and should certainly study its use before you begin. In any event, I think my results will challenge everyone's imaginations.

What Is Colchicine?

Colchicine is an alkaloid chemical derived from a flowering bulb of the lily family that is known as the autumn crocus (*Colchicum autumnale*). Plant breeders have long used colchicine in the development of new plant cultivars. (It has also been used in the treatment of the disease gout for over 2400 years.) Colchicine can make some plants produce larger flowers or tubers, although the effects are unpredictable. Also, colchicine can be used to restore fertility in sterile hybrids. While many carnivorous plant growers know about colchicine, there is little information about its application on carnivorous plants. Much of this article is based upon what I learned through experimentation.

How Colchicine Works

Colchine acts by disrupting cell plate formation during cellular division, so that as a plant cell divides into two cells it does not split. This produces a single cell with a doubled nucleus having twice the chromosome number. A plant with the chromosome number so increased by some multiple is known as a polyploid. Do not think of this as a corruption of nature. Polyploidy is a common natural occurrence, resulting from accidents in cell division. About half of all flowering plants have had origins in polyploidy (although the vast majority of *Drosera* are normal diploids with no evidence of polyploidy).

Hybrid plants are often sterile because the parent species have different chromosome numbers—as a result the chromosomes from the parents cannot pair compatibly. If such a hybrid has its chromosome number doubled, then the chromosomes are paired with their doubles. The genetic mechanics of the cell can then function more adequately and fertility is restored. The natural creation of such "allopolyploids" is an important process in the creation of new species because allopolyploids are sexually reproducing organisms. A prime example of a cultivated natural allopolyploid is the economically important bread wheat (*Triticum aestivum*), a hexaploid combination of three separate species. At least two sundews, perhaps more, evolved by means of allopolyploidy. In 1955 the botanist C.E. Wood (Wood, 1955) showed that *Drosera anglica* arose from the hybridization of two other species: *D. rotundifolia* (20 chromosomes) + *D. linearis* (20 chromosomes) = *D. angli-*

ca (40 chromosomes), although it was not proven that *D. linearis* was one of the parent species. I show a chromosome set diagram for this cross in Figure 1A. More recently (Nakamura & Ueda, 1991), scientists have found that the previously named *D. spatulata* 'Kansai' from Japan differed in chromosome number from the more typical form (60 chromosomes vs. 40 chromosomes, respectively) and is now called *D. tokaiensis*—it is probably another allopolyploid; *D. rotundifolia* (20 chromosomes) + *D. spatulata* (40 chromosomes)= *D. tokaiensis* (60 chromosomes).

What Can Be Done With Colchicine?

I have always been an avid tinker with my plants. Often I attempt cross-pollination experiments. Sometimes I have a clear picture in my mind of what kind of interesting new hybrid I want to achieve. But most times I just want to see what will happen. I have had several successes but far more failures. All this work tells me something, even the failures. Though I may not make something worth keeping, I do learn which plants are most closely related. In general, a sundew hybrid is either not formed at all after cross-pollinating two species with different chromosome counts, or the resultant hybrid is sterile and totally unable to produce seed. Fernando Rivadavia (another tinker) and I have found just a few exceptions to this general rule. We were surprised when the cross between *D. burmannii* from Australia and *D. sessilifolia* from Brazil proved to be fertile. (We are constantly debating about whether they should be considered separate species.) Amongst South African sundews, *D. dielsiana, D. nidiformis*, and *D. venusta* can all be crossed and produce fertile hybrids. Most *Drosera* hybrids involving species with the same chromosome count are perfectly fertile.

How Colchicine Is Applied

Colchicine is applied in various ways, and may be purchased as a powder (from chemical catalogs) or a premixed, ready to use paste (from a plant breeding supply company). When using the powder, the solution strength is not critical—I just mix colchine with enough water to dissolve it. In strawberry and other vegetable breeding a 1% solution is usually used. In every treatment method, the cells dividing in the meristematic growth point must be affected. If improperly treated, only a portion may be affected and the polyploidy will not continue throughout the plant. Seeds or entire seedlings can be soaked or it can be applied only to a plant's growth point. With orchids, colchicine is used in sterile tissue culture. For sundews it is best to treat leaf cuttings. I prepare cuttings on live sphagnum moss. I have found it is best to wait until tiny plantlets are sprouting. Soak the leaf cuttings (with tiny plantlets) in colchicine for 24 hours, using a sealed dark container (colchicine degrades in light). I next rinse off the cuttings and replace then on the sphagnum until they have grown to transplantable size.

Treated plants must be watched carefully to look for colchicine effects. When I treated the *D.* × *nagamotoi* (as described below), I obtained three plants. Two grew vigorously and looked healthy, while the third grew much more slowly and looked diseased. When the plants flowered I found that the weak plant was the only one that made seed, even though it did not make much pollen. Therefore, it was the only plant of the three made fertile by colchicine—the others were unmodified by the treatment. The seed from this plant produced seedlings with regained vigor. (Perhaps the newly polyploid nucleus is unstable and is stabilized by the act of reproduction.)

It is interesting that the first generation of allopolyploid *D.* × *nagamotoi* was so weak and slow-growing. This may be why more sterile sundew hybrids in nature do not spontaneously achieve fertility through amphiploidy to become new species as

D. anglica has done—perhaps the amphiploids are too weak to adequately reproduce themselves in nature. Perhaps only those natural hybrids which are not adversely affected by autopolyploidy have become new species, such as *D. anglica* and *D. tokaiensis*. Clearly; more research is required. I think this weak generation is also why others may have failed in using colchicine. A grower will automatically select the more vigorously growing plants and unknowingly dispose of the weaker plants which are the ones affected by the colchicine.

What Has Been Done So Far

I do not like sterile plants. (I discard all the sterile hybrids I make after my study of them is complete.) One exception to this is the natural hybrid called *D.* × *obovata*, a cross between *D. rotundifolia* and *D. anglica*. I have a specimen of this very attractive sundew as a living souvenir of a most wonderful camping and hiking adventure I had in July 1997 in northern California. I hoped to make this sterile plant into a fertile one so I could more easily spread it around to others as seed—it would be a thrill to see such a unique new plant offered in the ICPS seedbank. Unfortunately, all my attempts to produce a polyploid *D.* × *obovata* have resulted in weak plants (see Figure 1B). It looks like *D.* × *obovata* is an evolutionary dead end.

I have also treated a hybrid I made by crossing *Drosera anglica* (40 chromosomes) from Hawaii with a tetraploid *D. spatulata* (40 chromosomes) which I collected in Australia. Although I initially wanted to give this plant a new name, the parent species had previously been hybridized and officially named *D.* × *nagamotoi*, so that valid name must be used instead. I have been successful in changing *D.* × *nagamotoi* into a fertile octoploid with 80 chromosomes (see Figures 1C, 2). The octoploid has the highest chromosome count known for a sundew. Only one other species, *D. aliciae*, is reported to have this number but this high count is disputed (Bennett & Cheek, 1990). The flower of the allopolyploid *D.* × *nagamotoi* is white or pink. This sundew is not really better than anything that we already have, it is just something new. I have also made a fertile hexaploid *D.* × *nagamotoi* with 60 chromosomes (see the details given later in this paper). The most important feature of these plants is that they are the first manmade fertile "species." (Remember that it is much like the natural allopolyploid species *D. anglica*.) Even though these artificially enhanced crosses are interesting, they must still prove their benefit to horticulture.

In Table 1 I list most of the *Drosera* crosses I have attempted. It may be helpful for those who are studying the relationships of different species within the genus. Note, for example, that all the sterile hybrids of species with equal chromosome numbers listed involved *D. brevifolia*. Crosses I am making at the time of this writing are not included. For example, I am awaiting flowering of treated *D.* × *beleziana*. One of these is more densely tentacled, possibly a result of polyploidy.

Nomenclature and Taxonomy

This "new species" business is bound to bring about confusion and argument among those concerned with nomenclature. In my own notes I find it most convenient to simply list my first success as *D. nagamotoi* without the × symbol. Until a taxonomist officially publishes the name, the rule is that we must call it *D. anglica* × *spatulata*—fertile octoploid, or a little shorter *D.* × *nagamotoi*—octoploid. An even greater concern to taxonomists is the possibility that some unscrupulous breeder may try to pass off his own creation as a newly discovered species!

The latest trick I learned involves not doubling, but instead reducing chromosome numbers. While thinking about what would happen if a polyploid is crossed with a plant of a different ploidy, I developed a theory that I later learned has already been applied in breeding strawberries. The theory is basically that even-ploidy numbers in balanced allopolyploids—tetraploids, hexaploids, and octoploids—are fertile. Odd-ploidy numbers such as triploids are always sterile. I have found that this can be true regardless if they are made by doubling the chromosome number or by reduction. I have experimented along these lines with different polyploids of *D.* × *nagamotoi*. By crossing my octoploid *D.* × *nagamotoi* (80 chromosomes) with the tetraploid *D. anglica* (40 chromosomes) I created a hexaploid *D.* × *nagamotoi* (60 chromosomes), which is (by chromosome count) one third *D. spatulata* and two thirds *D. anglica*. This new hexaploid is fertile (see Figure 1D). This polyploid might also be made by crossing a diploid *D. spatulata* (20 chromosomes) from New Zealand with *D. anglica* (40 chromosomes) and then doubling the chromosomes with colchicine. The theory is not perfect—while my hexaploid *D.* × *nagamotoi* is fertile, it had totally defective pollen. In order to get seed I must use good pollen from another plant. But maybe I can use this plant for making something else.

I wonder what would happen if I crossed my new hexaploid *D.* × *obovata* (60 chromosomes) with *D. linearis* (20 chromosomes), but did not apply colchicine? The result would have 30 chromosomes from *D.* × *obovata* and 10 from *D. linearis* — that is, a tetraploid having a total of 40 chromosomes. This is much the same as *D. anglica* which has 40 chromosomes, half of which originate from *D. rotundifolia*

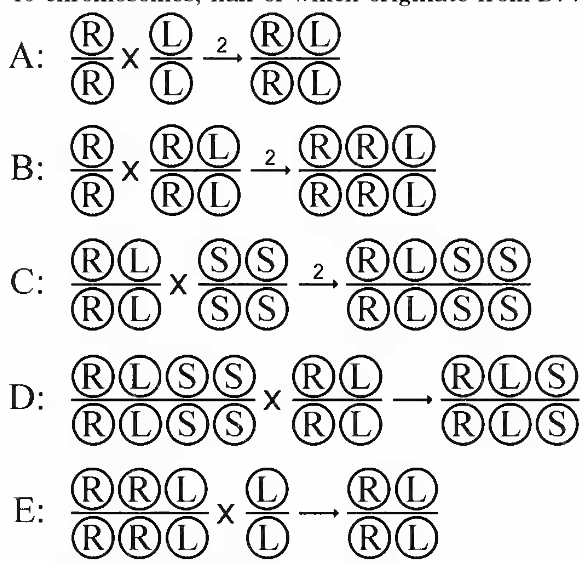

Figure 1: A) A chromosome set diagram showing how *D. rotundifolia* hybridized with *Drosera linearis*, followed by chromosome doubling via natural means (indicated by × 2) results in *Drosera anglica*. Each circled letter represents 10 chromosomes. B) Crossing *Drosera anglica* (see Figure 1A) with *D. rotundifolia*, followed by chromosome doubling via colchicine, resulted in a hexaploid *Drosera* × *obovata*. C) Crossing *Drosera anglica* (see Figure 1A) with a tetraploid *Drosera spatulata*, followed by colchicine doubling, resulted in a octoploid *D.* × *nagamotoi*. D) Crossing the octoploid *D.* × *nagamotoi* (see Figure 1C) with *Drosera anglica* resulted in a hexaploid *D.* × *nagamotoi*. E) Crossing the hexaploid *D.* × *obovata* (see Figure 1B) with *Drosera linearis* leads back to *Drosera anglica*.

and half from *D. linearis*. But would it be fertile? It seems as if it would be, if *D. anglica* was in fact half *D. rotundifolia* and half *D. linearis* (see Figure 1E).

What May Come in the Future

By using the diagrammatic theory, some very interesting new creations can be planned. I am now seeing new possibilities through crossings and selections of different polyploids. The colchicine-related deformities previously mentioned can be completely removed by crossing a treated plant with a normal untreated plant. Much more can be done with my octoploid *D.* × *nagamotoi*,and it is fun just to work out the permutations. Next I plan to cross different polyploids to see just how similar they must be in order to make fertile crosses. For instance, the hexaploids *D.* × *obovata* and *D.* × *nagamotoi* are both two thirds *D. anglica* and so differ by only one third (although, it should be noted that *D. anglica* is in turn one half *D. rotundifolia*, which is also the other parent of *D. obovata* but not of *D. nagamotoi*, so the first is in effect a back-cross, while the second is not.); will these make a fertile cross? Or perhaps plants differing by only one quarter can make a fertile cross? If this is possible then we can more easily mix and match traits of different species to produce novel cultivars, or recreate already existing species. I have crossed the hexaploid *D.* × *nagamotoi* with *D. rotundifolia*, reducing the chromosome number again to 40. This cross may be compatible with *D. anglica*. If this proves factual, then the pink flower from *D. spatulata* can be imported to *D. anglica*! In another direction, *D. tokaiensis* might be re-engineered by crossing the octoploid *D.* × *nagamotoi* with a tetraploid *D. spatulata*. The result would be a hexaploid that may be compatible with *D. tokaiensis*.

There is much more experimentation that needs to be completed, and this is only the beginning. I anticipate more successes. I hope that colchicine treatment

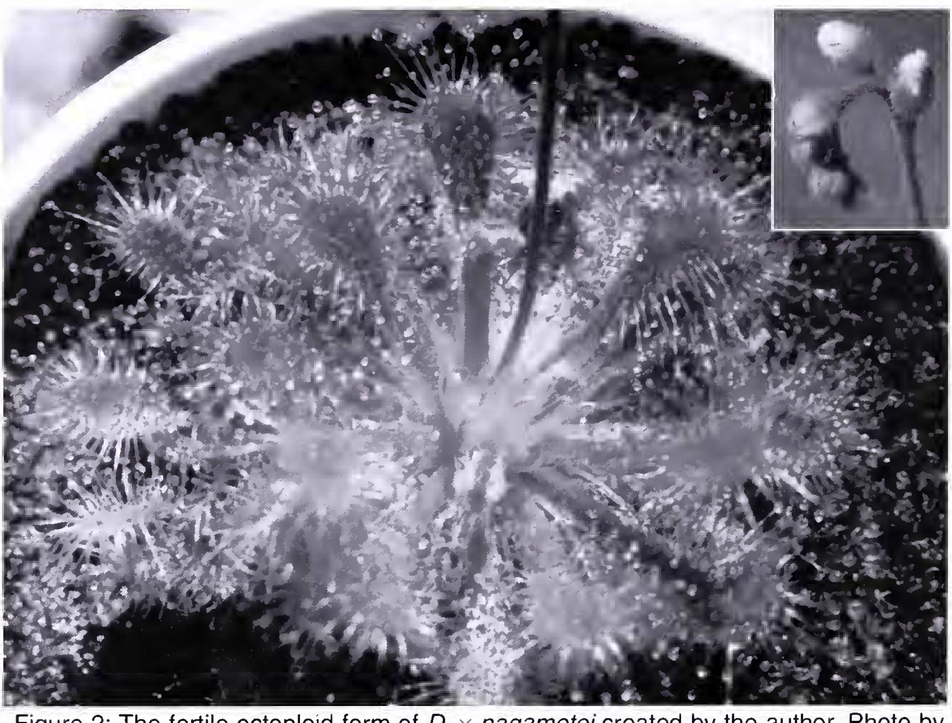

Figure 2: The fertile octoploid form of *D.* × *nagamotoi* created by the author. Photo by Edward Read.

Table 1: *Drosera* hybrids.

D. anglica × *indica*	B
D. anglica × *spatulata*	E
D. anglica × (× *nagamotoi*)	F
D. brevifolia × *aliciae*	D
D. brevifolia × *glanduligera*	B
D. brevifolia × *rotundifolia*	E
D. brevifolia × *spatulata*	E
D. brevifolia × *burmannii*	A
D. burmannii × *glanduligera*	A
D. burmannii × *sessilifolia*	G
D. capensis × *collinsae*	C
D. capensis × *dielsiana*	A
D. capensis × *spatulata*	B[1]
D. dielsiana × *nidiformis*	G
D. (*dielsiana* × *nidiformis*) × sp. Rhodesia	G
D. (*dielsiana* × *nidiformis*) × *spatulata*	D
D. esmeraldae × *anglica*	E
D. indica × *adelae*	A
D. madagascariensis × *spatulata*	A
D. montana × *brevifolia*	C
D. rotundifolia × *anglica*	E
D. rotundifolia × *brevifolia*	E
D. spatulata × *montana*	B
D. anglica—California × *anglica*—Hawaii	G
D. brevifolia—Louisiana × *brevifolia*—North Carolina	F
D. spatulata—typical Australian × *spatulata*—Gympie type	G

[1]Reported as F by others

The results of each cross are coded as follows. A—no seed or enlarged capsule produced; B—nonviable seed produced; C—seed viable but seedlings died; D—weak, sterile plants result; E—strong, sterile hybrids made; F—hybrids fertile, but with defective pollen; G—healthy, fertile hybrids.

can be as important a tool in the breeding of our interesting carnivorous plants as it has for flowers and vegetables. At the time of writing, all my creations have been small sundews. Larger plants would be more popular. I have just begun working on crossing my tropical *D. anglica* with the Florida *D. filiformis*. I may get some large plants having no dormancy requirement. This, when treated with colchicine, will produce a hexaploid which might possibly make a fertile cross with *D.* × *nagamotoi*. This in turn would lead to a high degree of variability for much more future selection. Watch for the diabolical manmade sundew species!

Before you begin to design your own new species, here is a helpful hint. I learned that it is best to start working with easily grown and freely flowering hybrids. The "tropical" *D.* × *obovata* I finally made was by using the more easily grown Hawaiian form of *D. anglica* crossed with *D. rotundifolia*. The natural *D.* × *obovata* I had first treated was too weak to sufficiently produce a hibernaculum, as it must do before flowering.

Much selecting must be done to sort out the strongest seedlings. Expect deformities; my *D.* × *nagamotoi* has ragged petals. Some hybrids will be too malformed to be of value. All hybrids may not be so adversely affected by colchicine. *D.* × *beleziana* does not seem to exhibit much deformity, while *D.* × *obovata* is extremely malformed and not worth reproducing.

I wish anyone else thinking of undertaking a treatment project good luck. It is fun just to think of the possibilities. More people will likely want to simply cross-breed the different polyploids rather than use a dangerous chemical. I do hope to see others produce some outstanding new cultivars through the use of colchicine.

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Colchicine Hazards

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Keywords: Genetics: colchicine.

(While Ivan Snyder's experiments are exciting, we wish to ensure that the biohazards associated with colchicine are clearly understood by readers of Carnivorous Plant Newsletter. We invited Sean Barry, from the University of California, to comment on the compound—BAMR)

Colchicine is by far the most dangerous chemical that home-based cell culturists might encounter in their experiments. It is part of a disparate group of chemicals that are capable of altering genetic material, in this case by disrupting the mitotic spindle that aligns and "tracks" the chromosomes during cell division. Cell division is thus arrested, and the result is polyploidy, or multiple sets of chromosomes (>2) within a single cell. This is potentially desirable in plant genetic engineering, but extremely hazardous if it affects certain tissues in the user, particularly germ cells (sperm and ova) and the developing embryo. For this reason, colchicine is classed as a teratogen (a substance that causes birth defects) and may also be a potential carcinogen. Colchicine is also very toxic. A single oral dose of as little as 3.0 milligrams (that is 0.003 grams, or 0.0001 ounce) has caused death, and the rat LD50 (i.e. the dosage that is lethal to about 50% of an experimental group) is as little as 0.125 milligrams per kilogram of body weight (and is presumably comparably toxic to humans). The material is equally toxic when ingested, inhaled as powder, or absorbed through the skin. Potential users of colchicine should first be trained in proper storage, handling and personal protection measures, and they should observe state and local disposal regulations.

References: Mallinckrodt-Baker and Abbott Labs Material Safety Data Sheets for colchicine reagent and pharmaceutical preparations.